FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **BMID9812US**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/806983**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/07366	**(05.10.99) 5 October 1999**	**(08.10.98) 8 October 1998**

TITLE OF INVENTION
METHOD FOR DETERMINING ALKALINE PHOSPHATASE

APPLICANT(S) FOR DO/EO/US
WEISHEIT, Ralph; and TREIBER, Wolfgang

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment. (to follow)
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

General Appointment of Representative for U.S. Patent and Trademark Office Matters; and Return postcard.

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/806983	PCT/EP99/07366	BMID9812US

21. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO	$970.00
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO	$840.00
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$690.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$670.00
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	$96.00
	ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	- 20 =	0	x $18.00	$0.00
Independent claims	- 3 =	0	x $78.00	$0.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
	TOTAL OF ABOVE CALCULATIONS		=	$860.00
	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.		☐	$0.00
	SUBTOTAL		=	$860.00
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).		+	$0.00
	TOTAL NATIONAL FEE		=	$860.00
	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**		☐	$0.00
	TOTAL FEES ENCLOSED		=	$860.00
			Amount to be: refunded	$
			charged	$

☐ A check in the amount of to cover the above fees is enclosed.

☒ Please charge my Deposit Account No. **50-0877** in the amount of **$860.00** to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **50-0877.** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

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6 April 2001
DATE

Method for determining alkaline phosphatase

The present invention concerns a method for the determination of alkaline phosphatase in a sample by optical measurement which is characterized in that a main measurement wavelength of 450 ± 10 nm in combination with the rate blank method is used to eliminate haemoglobin interference, a method for eliminating interference by free haemoglobin or blood substitutes and the use of the combination of a main measurement wavelength with the rate blank method to eliminate interference by free haemoglobin or blood substitutes.

It is known that haemolysis considerably interferes with some diagnostic methods for the determination of analytes. Haemolysis is understood as any destruction of erythrocytes for example by mechanical, osmotic, chemical or enzymatic action on the cell membrane of the erythrocytes. As a result of haemolysis, the blood pigment haemoglobin (Hb) is released and can no longer be removed from a sample. The presence of haemoglobin is problematic because, on the one hand, the absorption spectrum of haemoglobin in some cases overlaps considerably with the spectra of the substances to be detected and indicators (chromogens) which can result in measuring errors in photometric tests. On the other hand, haemoglobin can also react chemically with sample components to form substances which can also result in false measurements.

Recently blood substitutes whose manufacture is based on haemoglobin are being used more and more frequently for therapeutic purposes for example after a large loss of blood. The haemoglobin in blood substitutes can be native or synthetic. Often Hb-like compounds are also used. In contrast to haemolysis in which there is usually a haemoglobin content of up to 500 mg/dl, the Hb content in blood serum or plasma may be more than 2000 mg/dl during treatment with blood substitutes. Hence interference in samples which contain blood substitutes is often considerably more pronounced than in haemolytic samples since the haemoglobin or the synthetic analogue is in a free form right from the beginning.

Interference by free haemoglobin is particularly serious in the photometric determination of alkaline phosphatase. The formation of 4-nitrophenol is measured at 405 to 415 nm (increase of absorbance) for the determination of alkaline phosphatase. Haemoglobin also absorbs at 415 nm. The presence of haemoglobin interferes with the determination of alkaline phosphatase in two respects: On the one hand the Hb spectrum changes in a time-dependent manner (increase of absorbance) in an alkaline medium, on the other hand, the photometer limit of the measuring instrument is reached above a certain Hb content.

Various methods have been published in the prior art to eliminate the spectral and chemical influence of haemoglobin on the analysis of serum or plasma samples.

Jay and Provasek describe in Clin. Chem. 39/9, 1804-1810 (1993) that haemoglobin interference of the alkaline phosphatase determination is caused by a time-dependent

change of the Hb spectrum. This interference can be eliminated by mathematical correction algorithms (determination of the Hb concentration in the sample and correction of the measured value for alkaline phosphatase by a certain amount that is equivalent to the measured amount of Hb).

Although the mathematical correction mentioned by Jay and Provasek eliminates the influence of Hb up to at least 800 mg/dl Hb, it is, however, not very user-friendly since it requires an additional measurement of the Hb content and subsequently an additional mathematical correction step.

Jay and Provasek (supra) describe a further method for eliminating interference by the so-called rate-blank measurement. The correction of haemolysis interference by rate-blank measurements is also described in EP-A-0 695 805. In this method the sample is subjected to a pre-reaction to determine the degree of haemolysis of the sample before the actual photometric determination of a component contained in the sample. The measured value obtained subsequently is then corrected by a value which has been determined by correlating the degree of haemolysis with the amount by which the interfering components contribute to the measuring error.

Hb interference can be eliminated by rate-blank measurements but only up to a Hb content of ca. 1200 mg/dl since the photometer limit is reached at higher Hb contents. This may be adequate for eliminating haemolysis interference but it is not sufficient at all for eliminating interference by blood substitutes.

Another method for eliminating haemoglobin interference was published for the determination of albumin (PCT application WO 97/45728) in which an elimination of haemoglobin interference was achieved by special combinations of main and secondary wavelengths. However, the wavelength combinations mentioned here cannot be used for the determination of alkaline phosphatase since a measuring signal would no longer be obtained for 4-nitrophenol at these wavelengths.

The laid-open publication WO 97/45733 describes that interference by haemoglobin can be eliminated by using the secondary wavelengths 546 and 570 in individual UV tests. However, this method can only be used for enzymatic UV tests with a main measurement wavelength of 340 nm. Although a complete elimination of Hb interference can be achieved solely by the use of the secondary wavelengths 546 or 570 nm, this is not possible for enzymatic chromogenic tests such as the determination of alkaline phosphatase in which the main measurement wavelength is in the range of 415 nm.

The US patent 5,766,872 mentions that a secondary wavelength of 577 nm reduces haemolysis interference in the amylase determination. However, the quoted measurement data show that there is already a significant deviation of the measured values of up to 8 % at a Hb content of 500 mg/dl. This may be sufficient to eliminate haemolysis interference but it is probable that at higher Hb concentrations (such as those which occur during treatment with blood substitutes) this deviation of the measured values would become larger due to the use of a main measurement wavelength of ca. 415 nm and that there would no longer be an adequate elimination of Hb interference.

No method for the determination of alkaline phosphatase is known in the prior art which can also be carried out without interference in the presence of high concentrations of Hb such as those which occur in samples containing blood substitutes.

The object was therefore to develop an improved method for the determination of alkaline phosphatase in a sample which largely overcomes the disadvantages of the prior art. In particular it is intended to provide a simple and user-friendly method for eliminating interference by haemoglobin and by blood substitutes based on haemoglobin when determining alkaline phosphatase.

The object is achieved by a method described in more detail in the claims for the determination of alkaline phosphatase in a sample by optical measurement. The method is characterized in that 450 ± 10 nm is used as a main measurement wavelength in combination with the rate blank procedure.

It surprisingly turned out that Hb interference of the determination of alkaline phosphatase can be effectively eliminated when the main wavelength is changed and the rate blank procedure is used. It is not sufficient for a satisfactory elimination of Hb interference to only change the main wavelength or only use the rate blank procedure.

Due to the absorption spectrum of 4-nitrophenol it is possible to measure alkaline phosphatase not only at 415 nm but also at 450 ± 10 nm. Although the main measurement wavelength is then not in the usual

absorption maximum of the detection reaction but on its flank, the measured signal obtained is nevertheless adequate for an exact determination of alkaline phosphatase.

The selection of the new main measurement wavelength of 450 ± 10 nm already leads to a slight reduction of the haemoglobin interference, but a complete elimination of interference is surprisingly only obtained by combining the main wavelength of 450 ± 10 nm with the rate blank procedure.

The method according to the invention enables interference of the alkaline phosphatase determination by haemoglobin or haemoglobin-like compounds to be eliminated for the first time in a simple manner up to a Hb content of at least 3000 mg/dl. The upper limit for the elimination of Hb interference is the limit determined by the performance of the photometer. Hence the method according to the invention can be expected to achieve a good elimination of interference up to 6500 mg/dl haemoglobin content.

In the rate blank procedure according to the invention the sample is subjected to a pre-reaction to determine the degree of haemolysis of the sample before the actual photometric determination of a component contained in the sample. The measured value for the component to be determined obtained subsequently is then corrected by a value which has been determined by correlating the degree of haemolysis with the amount by which the interfering components contribute to the measuring error. Use of the rate blank procedure per se to correct for haemolysis interference is described for example in

EP-A-0 695 805 and by Jay and Provasek in Clin. Chem. 39/9, 1804-1810 (1993).

The secondary measurement wavelength used for the rate blank procedure is unimportant for the invention. Also the length of the time window for the measurement of the pre-reaction and the main reaction is not decisive. It has proven to be suitable to measure the absorbance change of the pre-reaction and main reaction over a time period of 1 to 4 minutes.

The method according to the invention is suitable for a determination of any samples in which free haemoglobin is present. The term free haemoglobin in the sense of the invention is used to distinguish it from haemoglobin which is present in intact erythrocytes. Examples of samples which contains free haemoglobin are haemolytic serum or plasma samples or samples which contain blood substitutes. Examples of blood substitutes that fall under the term free haemoglobin in the sense of the present invention are derivatized, polymerized, modified or cross-linked derivatives of haemoglobin in particular human haemoglobin or bovine haemoglobin e.g. DCL haemoglobin (diaspirin-crosslinked haemoglobin) or recombinantly produced haemoglobin.

The invention also concerns a method for eliminating interference caused by free haemoglobin in a method for determining alkaline phosphatase. The method is characterized in that a main measurement wavelength of 450 $\pm$ 10 nm in combination with the rate blank procedure is used.

A further subject matter of the invention is the use of a main measurement wavelength of 450 ± 10 nm in combination with the rate blank procedure to eliminate interference by free haemoglobin or by blood substitutes manufactured from haemoglobin in a method for determining alkaline phosphatase.

The invention is elucidated by the following example.

Example

a) Preparation of samples containing haemoglobin

A solution containing Hb was added to a part of a serum pool to yield a Hb content of at least 3000 mg/dl. Another part of the same serum pool of the same volume was admixed with an equivalent amount of NaCl solution (154 mmol/l). Both parts were subsequently mixed with one another in different ratios to obtain a Hb concentration series of 11 samples with no Hb in the lowest sample and at least 3000 mg/dl Hb in the highest sample.

b) **Determination of alkaline phosphatase according to the SFBC method**

Determination according to the recommendation of the Société Française de Biologie Clinique according to Ann. Biol. Clin. Vol. 35, 271 (1977)

The determination of alkaline phosphatase was carried out on a Boehringer Mannheim/Hitachi 911 analyzer.

The following reagents were used:
reagent 1: 930 mmol/l 2-amino-2-methyl-1-propanol buffer
pH 10.5;
1.03 mmol/l magnesium aspartate
reagent 2: 930 mmol/l 2-amino-2-methyl-1-propanol buffer,
pH 10.5;
1.03 mmol/l magnesium aspartate;
98 mmol/l 4-nitrophenyl phosphate

The test procedure was as follows: 250 µl reagent 1 was added to 11 µl sample and after 5 min 50 µl reagent 2 was added. For the comparative measurements the analyte was determined after a further 50 sec during which the change in absorbance was measured during the subsequent 4 min. Combinations of the following main measurement wavelengths (λ_1) and secondary measurement wavelengths (λ_2) were used for the measurement: λ_1/λ_2 = 415/660 nm (previous instrument settings), 415/570 nm and 450/660 nm (comparison). Furthermore alkaline phosphatase was determined by the rate blank measurement mentioned by Jay and Provasek as a further comparison (referred to as 415/660 nm RB).

The measurement wavelength combination λ_1/λ_2 = 450/660 nm was used to determine the analyte according to the invention. For the rate blank measurement the change in absorbance of the pre-reaction was measured in the period 3.0 - 4.9 min after addition of reagent 1 to the sample and the change in absorbance of the main reaction was measured in the period 7.9 - 9.8 min after addition of reagent 1 in the sample. This corresponds to the measurement points [10] - [16] and [25] - [31] on the Boehringer Mannheim/Hitachi 911 analyzer. The result is shown in the column labelled "450/660 nm RB".

The results of the measurement according to the invention as well as the comparison measurements are shown in the following table 1. It can be seen that the use of the inventive combination of the new main wavelength of 450 nm and the rate blank procedure considerably reduces, interference by blood substitutes containing Hb compared to the other measurement wavelength combinations or compared to the rate blank measurement at the main wavelength of 415 nm.

Table 1

Measured content of alkaline phosphatase at 37°C in U/l

Hb content* [mg/dl]	415/660 nm	415/570 nm	450/660 nm	415/660 nm RB	450/660 nm RB
0	42	42	42	42	42
300	32	32	35	44	43
600	22	24	29	46	44
900	14	16	24	46	46
1200	8	11	22	44	45
1500	3	7	19	5	47
1800	-2	2	17	0	47
2100	-2	3	17	0	48
2400	-2	3	15	1	50
2700	-1	3	16	0	49
3000	-2	3	19	1	51

* in this case a cross-linked haemoglobin was used.

Claims

1. Method for the determination of alkaline phosphatase in a sample by optical measurement, wherein a main measurement wavelength of 450 ± 10 nm is used in combination with the rate blank procedure.

2. Method as claimed in claim 1, wherein the determination is carried out in a serum or plasma sample.

3. Method as claimed in one of the previous claims, wherein a sample is determined which contains free haemoglobin or a blood substitute manufactured on a haemoglobin basis.

4. Method as claimed in one of the previous claims, wherein the blood substitute contains a derivatized, modified or cross-linked human haemoglobin, bovine haemoglobin or a recombinantly produced haemoglobin.

5. Method as claimed in one of the previous claims, wherein the sample has a haemoglobin content of up to 6500 mg/dl.

6. Method for eliminating interference caused by free haemoglobin or blood substitutes in a method for determining alkaline phosphatase, wherein a main measurement wavelength of 450 ± 10 nm is used in combination with the rate blank procedure.

7. Use of a main measurement wavelength of 450 ± 10 nm in combination with the rate blank procedure to eliminate interference by free haemoglobin or by blood substitutes in a method for determining alkaline phosphatase.

Abstract

The invention concerns a method for the determination of alkaline phosphatase in a sample by optical measurement which is characterized in that a main measurement wavelength of 450 ± 10 nm in combination with the rate blank procedure is used to eliminate haemoglobin interference, a method for eliminating interference by free haemoglobin or blood substitutes and the use of the combination of a main measurement wavelength with the rate blank procedure to eliminate interference by free haemoglobin or blood substitutes in the determination of alkaline phosphatase.

Docket No.

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

METHOD FOR DETERMINING ALKALINE PHOSPHATASE

the specification of which

(check one)

☐ is attached hereto.

☒ was filed on October 05, 1999 as United States Application No. or PCT International Application Number PCT/EP99/07366

and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
198 46 300.6 (Number)	DE (Country)	08 October 1998 (Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Assistant Commissioner for Patents
Washington, DC 20231

GENERAL APPOINTMENT OF REPRESENTATIVE FOR U.S. PATENT AND TRADEMARK OFFICE MATTERS

The undersigned applicant or assignee hereby appoints D. Michael Young, Reg. No. 33,819, Richard T. Knauer, Reg. No. 35,575, Brent A. Harris, Reg. No. 39,215, Kenneth J. Waite, Reg. No. 45,189, and Marilyn L. Amick, Reg. No. 30,444 all of Roche Diagnostics Corporation, 9115 Hague Road, P.O. Box 50457, Indianapolis, Indiana 46250, Telephone No. (317) 845-2000, and Jill Lynn Woodburn, Reg. No. 39,874 of The Law Office of Jill L. Woodburn, L.L.C., 6633 Old Stonehouse Drive, Newburgh, Indiana 47630-1785, Telephone No. (812) 842-2660:

to prosecute and transact all business on its behalf before the United States Patent and Trademark Office in connection with any U.S. patent assigned to it and any U.S. patent application filed by it or on its behalf and to receive payments on its behalf.

Signed this 18th **day of** September **, 2000 at Mannheim, Germany.**

Roche Diagnostics GmbH

Richard Jung
Signature

Dr. Michael Jung
Print Name

Director
Position or Title

Roche Diagnostics GmbH

Manfred Weber
Signature

Dr. Manfred Weber
Print Name

Senior Director
Position or Title

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